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RAPD analysis of genetic diversity of nine strains of *Auricularia auricular* cultivated in Heilongjiang Province

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Abstract: Polymorphism of nine strains (CF05, CF09, 29, 916, AU9, Chang10, Chang7, 8808 and AU. Japanese) of *A. auricular* cultivated in Heilongjiang Province were analyzed by RAPD (Random Amplication polymorphic DNA). Thirteen primers were selected from forty PCR primers with 10bp long random primer. The results showed that nine strains of *A. auricular* have a high level of genetic diversity and the percentage of DNA polymorphic was 96.05. The genotypes of 9 strains of *Auricularia auricular* were identified by the fingerprints from primer 27 and primer 46 by RAPD analysis. The results are helpful for quickly identifying strains of *A. auricular* in its early breeding time, and also provides a powerful theoretic basis to differentiate strains (*Auricularia auricular*) whose morphology is very similar in breeding programs of edible fungus.

Keyword: *Auricularia auricular*; RAPD analysis; DNA fingerprint; Genetic diversity

Introduction

Auricularia auricular, as one kind of valuable nourishment fungus, has richer pectin and it is a kind of natural and healthful food. Due to the lack of useful criteria in distinguishable character, it is difficult to differentiate the commercial cultivated strains in the breeding *A. auricular* strains. For example, the same strain may has different names, or the same name was used for different strains (Yan *et al*. 2000). In recent years, RAPD technique has been used to identify varieties of crops and determine their genetic relationship between varieties, and this technique is also useful for gene mapping in identifying strains of edible fungus (Wu *et al*. 2004; Zeng *et al*. 2001; Zeng *et al*. 2001; Yan *et al*.1999; Ma *et al*.2002). In this study, RAPD technique was used to differentiate the strains of *A. auricular* cultivated by analyzing their genetic diversity and RAPD fingerprints of different strains (Zhang *et al.*1999; Yuan *et al*.2000; Wu *et al*. 2002). This study can be helpful for distinguishing of *A. auricular* strains in DNA molecular level.

Materials and methods

Materials

Nine strains (CF05, CF09, 29, 916, AU9, Chang10, Chang7, 8808 and AU. Japanese) of *A. auricular* were reserved in microbiological laboratory of Northeast Forestry University. Strains of 29, 916, CF05, CF09 and 8808 were provided by the Applied Microbiology Institute of Heilongjiang, strains of Chang 7 and Chang 10 were separated and domesticated from wild *A.auricular* in Changbai Mountains, strain of AU. Japanese was introduced from Japan, and strain of AU9 was separated from wild *A. auricular* by Youhao Fungus Institute in Heilongjiang Province, Yichun.

DNA extraction and purity examination

The DNA marker, dNTP, Buffer, Primer and Taq DNA polysenthsis enzymes were obtained from Takara Company. Strains of *A. auricular* were cultivated in the PD (potato and dextrose) liquid medium under 25℃ at 200 rpm for 10 days in shaking incubator. DNA was extracted from the mycelium by ordinary method of Ethanol-chloroform. Purity of extracted genomic DNA was examined by UV spectrophotometer and the gel with 1.5% agarose.

RAPD-PCR analysis

Primer sequence

Thirteen primers were screened out from 40 random PCR primers. Their sequences were marked and given in Table 1.

PCR reaction system and its parameter

The total volume of amplification reaction solution was 15μL, which was consisted of 1.5 μL of 10×Buffer (Mg^{2+}), 0.6 μL of 2.5mM dNTP, 0.6 μL of Primer, 0.2 μL of 5U/μL TaqE, 3−4μL of 2mM DNA template and ddH_2O. The PCR reaction was performed in shaking incubator by following parameters: predenaturing for 5 min at 94 ºC, denaturing for 60 s at 94 ºC, annealing for 60 s at 36 ºC and extension for 2 min at 72 ºC and then 40 cycles and extension for 10 min at 72 ºC in shaking incubator at last. Amplified products of *A. auricular* genomic DNA were separated by eletrophoresis in gel with 1.5% agarose and stained with EB ($0.5mg \cdot L^{-1}$). Polymorphisms were observed and photoed under ultraviolet light.

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Table 1. Primers for RAPD amplification and their nucleotide sequences

Primer code	Sequence	Primer code	Sequence
P26	GGA CTG GAG T	P50	GTG ACG TAG G
P27	TGC GCC CTT C	P52	GTG ATC GCA G
P32	CTG CTG GGA C	P55	CAG CAC CCA C
P33	GTA GAC CCG T	P56	TCT GTG CTG G
P45	AGT CGA CCA C	P57	TTC CGA ACC C
P46	AAT CGG GCT G	P60	AGG TGA CCG T
P49	GAA ACG GGT G		

Data analysis

The genetic diversity of strains of *A. auricular* was analyzed by DNA fingerprints. The DNA bands at same molecular weight are known as common band, which means that they come from the same DNA loci. The same DNA loci means that the band in DNA fingerprints is at one site and no polymorphism. The bands at difference molecular weight mean that they are special DNA bands to have polymorphism. The obvious band displayed was marked as "1", and the unobvious band and no band were marked as "0". All of the DNA bands are shown according to their molecular weight (Fig. 1). Resembled index and genetic distance index (Table 3) are calculated by Nei method (1972). Phylogenetic tree was constructed by SPSS (v11.5) with UPGMA method. **(**Fig. 3)

Results

RAPD-PCR fingerprints analysis

The stable DNA fingerprints of 9 strains were got from 13 random primers screened out. One hundred and seventy nine DNA bands were got from 13 random primers, of which there were 172 special bands (Table 2). The molecular weights for different bands ranged from 0.75kb to 2.0 kb. The signed number of bands from single primer changed from 0 to 13.

Table 2. PCR results of 13 random primers for RAPD analysis

No.	Primer code	Band number of single primer	Total bands	Polymorphism band number
1	P26	0-13	16	16
2	P27	3-9	13	11
3	P32	4-9	16	15
4	P33	2-9	17	17
5	P45	3-10	14	13
6	P46	2-6	13	12
7	P49	1-10	12	12
8	P50	0-6	14	14
9	P52	2-4	11	11
10	P55	5-11	14	12
11	P56	2-8	11	11
12	P57	0-9	12	12
13	P60	3-9	16	16
Total			179	172

Strains identification

A comparison between 13 random primers on PCR showed that every strain had its special DNA fingerprints. Fingerprints of primer 27 showed that 9 strains had total 13 bands, of which DNA bands at molecular weights for 1.00kb, 1.25kb and 1.70kb were three common bands. Fingerprints form primer 46 also had 13 DNA bands but there was only a common band at molecular weight for 0.06kb (Fig. 1A and B). The nine strains of *A.auricular* were clearly distinguished each other by dendrogram of identification molecular weight with Primer 27 and Primer 46 (Fig. 2).

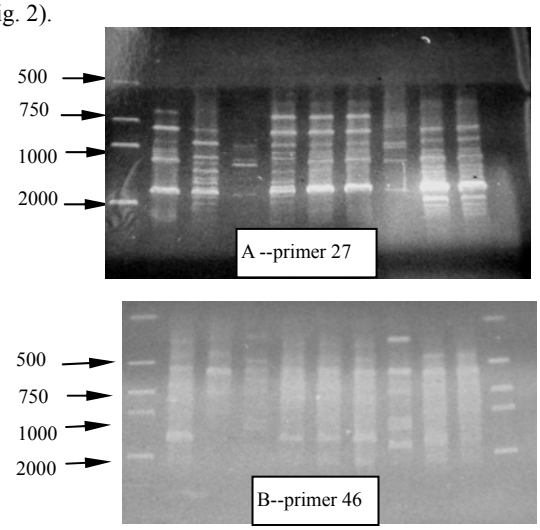

Fig. 1 Fingerprint for 9 strains of *Auricularia auricular* by primer 27 (A) and primer 46 (B)

Genetic distance index and genetic similarity index

DNA bands of RAPD-PCR from 13 primers were grouped by cluster analysis. The genetic distance index for nine strains was from 0.9324 to 0.2016. This meant their genetic distance indexes changed from 0.0676 to 0.7984. The average genetic similarity index of 9 strains of *Auricularia auricular* was 0.5122 and the average genetic distance index was 0.4878 (Table 3). The highest genetic similarity was found between the strain 8808 and Chang10 and the lowest genetic similarity was found between the strain 916 and AU.Japanese.

Genetic relation and clustering analysis

Genetic relation and clustering analysis showed that there was a close genetic relationship between the 8808 and Chang 10, Chang 7 and CF05 and Au.Japanese with Au9. Au.Japanese and Au9 had the farthest genetic relative relationship with other strains (Fig. 3).

Discussion

Nine strains of *A. auricular* were analyzed by RAPD-PCR. The genetic similarity was lower (average value 0.5) within nine strains but there were rich genetic diversity (Table 3). Therefore, these strains can be used as a resource for breeding other strains. The strains of Au.Japanese and 916 had a further relative relationship due to having different origins of strains. The phylogenetic tree of genetic distance index and clustering analysis can explained the genetic relative relationship among nine strain of *A. auricular* (Fig. 3). Both the genetic similarity index and dendrogram of identification molecular showed that strains of Chang 10 and 8808 have the nearest genetic relative relationship. The nine

strains of *A. auricular* were identified each other by using different bands in Primer 27 and Primer 46.

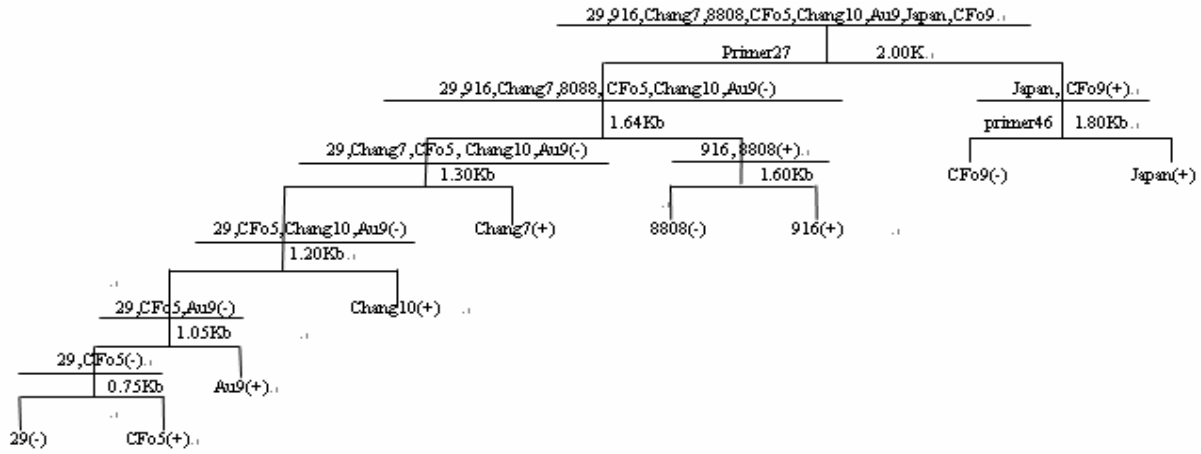

Fig. 2 Dendrogram of identification molecular weight for 9 strains of *Auricularia auricular*

（+）represented that there was DNA fragment in the molecular weight ;（-）represented that there was no DNA fragment in the molecular weight.

Table 3. Matrix of genetic similary index and genetic distance index of nine strains of *Auricularia auricular*

	29	CF09	AU.J	916	Chang7	CF05	AU9	Chang10	8808
29		0.4037	0.7600	0.1868	0.1474	0.1579	0.6324	0.5432	0.5244
CF09	0.5963		0.7593	0.2552	0.3873	0.3873	0.6320	0.3485	0.2794
AU.J	0.2400	0.2407		0.7984	0.7956	0.7956	0.6327	0.7615	0.7477
916	0.8132	0.7448	0.2016		0.0825	0.0825	0.6924	0.4675	0.4524
Chang7	0.8526	0.6127	0.2044	0.9175		0.0693	0.6687	0.5172	0.4545
CF05	0.8421	0.6127	0.2044	0.9175	0.9307		0.6687	0.5402	0.4773
AU9	0.3676	0.3680	0.3673	0.3076	0.3313	0.3313		0.6889	0.6934
Chang10	0.4568	0.6515	0.2385	0.5325	0.4828	0.4598	0.3111		0.0676
8808	0.4756	0.7206	0.2523	0.5476	0.5455	0.5227	0.3066	0.9324	

Notes: Numbers in the upper triangle are genetic distance index and in the lower triangle is genetic resemble index.

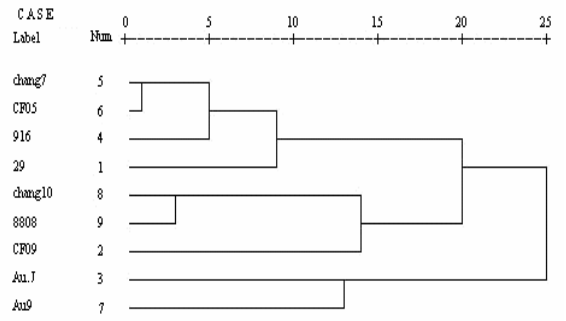

Fig. 3 Phylogenetic tree constructed with UPMGA of 9 strains of *Auricularia auricular*

It was very important to establish the identification method in commercial edible fungus. Studies showed that RAPD was a quick and accurate method in identifying the strains of edible fungus. The random primers screened in this study can be used to identify the other strains of *A. auricular*. Therefore, the RAPD analysis of genetic diversity of nine strains of *A. auricular* will provide the useful information for breeding commercial strains.

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